Supporting Online Material for

Wolbachia enhance stem cell proliferation and target the germline stem cell niche

Eva M. Fast, Michelle E. Toomey, Kanchana Panaram, Danielle Desjardins Eric D. Kolaczyk and Horacio M. Frydman*

*correspondence to: hfrydman@bu.edu

This PDF file includes:

Materials and Methods
SOM Text
Figs. S1 to S9
Tables S1 to S6
Captions for Movies S1
Supplementary References 32 to 57

Other Supporting Online Material for this manuscript includes the following:

Movies S1

Contents

Materials and Methods

Fly stocks and experimental husbandry conditions

The *Drosophila mauritiana* fly stock was a kind gift from Virginie Orgogozo and Maria Margarita Ramos (David Stern Lab, Princeton University). Presence of *Wolbachia* infection in this stock was confirmed by immunocytochemistry and PCR. Sequencing of the WSP gene revealed homology to *w*Mau sequence (32). The *Wolbachia* non-infected (W-) stock was generated by treatment of the *Wolbachia*-infected (W+) stock for two generations with tetracycline (0.025% w/v) (33). In order to homogenize the nuclear genetic background, W- females were backcrossed to W+ males for 3 generations (see Supplementary Figure 1 and Supplementary Reference 34)

Fecundity tests were done similarly to Drummond-Barbosa and Spradling 2001 (35). Fifteen newly eclosed W+ and W- flies were collected and kept in three bottles containing yeasted apple juice agar plates (5 females and 5 males per bottle). Apple juice plates were changed every 24 hours and numbers of eggs laid were counted. For the fecundity experiment # 1 (RT), flies were raised at room temperature (RT, fluctuating around 22°C, and humidity varying from 20-40%). In experiment 2 (25°C), flies were kept for several generations in a 25°C incubator (60% humidity). We did not observe a significant difference for *Wolbachia* levels between 22°C and 25ºC.

For the assessment of programmed cell death in the germarium and rate of stem cell division, flies were raised and kept in a 25°C incubator (60% humidity). Newly eclosed females were collected and kept with an equal number of males in yeasted vials for ~24 hours after which they were dissected and processed for staining. For the analysis of the later stage PCD checkpoint, flies were kept for two days in yeasted vials. The vials were changed once after 24 hours.

Immunohistochemistry

Ovarioles were stained as previously described (36, 37). The following antisera were used at the indicated dilutions: anti-hsp60 (Sigma) (1:100), rat anti-vasa (a gift from P. Lasko; 1:500), rabbit anti-phosphorylated histone 3 (1:200, Upstate Biotech), rat anti-α-catenin (1:40, DSHB), mouse anti-BrdU (1:100, Amersham, RPN20AB), rabbit anti-Alpha Spectrin (38)(1:500, kind gift from Trudi Schüpbach). Nuclei were counterstained with Hoechst (1 µg/ml, Molecular Probes).

Bromodeoxyuridine (BrdU) labeling

BrdU staining was done according to Lilly and Spradling (39). Ovaries of W+ and W- flies were dissected in alternating order in Grace's medium complemented with L-Glutamine (Cambrex) and incubated in the same media containing BrdU (0.5 mg/ml, Sigma) for 1.5 hrs. After a quick wash, ovaries were fixed with 4% Paraformaldehyde. To expose incorporated BrdU for antibody detection, ovaries were treated with DNAse (Promega, 25U per sample) for 30 min at 37°C. Subsequently, BrdU immunostaining was performed.

TUNEL labeling

One of the hallmarks of programmed cell death is DNA fragmentation which can be detected using the TUNEL (**t**erminal deoxyribonucleotidyl transferase d**U**TP **n**ick **e**nd

labeling) assay. For this purpose the ApopTag fluorescein direct in situ Apoptosis Detection Kit (S7160, Chemicon) was used. Dissected and fixed ovaries were washed twice with equilibrium buffer for 5 minutes at room temperature and then incubated overnight at 37°C in TdT solution. Ovaries were washed for 5 minutes in stop/wash solution followed by immunostaining to perform double labeling for *Wolbachia*.

Acridine Orange

For live imaging of PCD based on Acridine Orange incorporation, ovaries of W+ and W- females were dissected in alternating order in Grace's medium complemented with L-glutamine, then incubated in Acridine Orange solution (10 μg/ml) for 5 minutes at RT. Ovaries were rinsed three times with PBS and mounted in Halocarbon oil 700 for immediate analysis with a spinning disk confocal microscope (Olympus). Ovaries were analyzed in alternating order between W+ and W- to standardize the period between dissection and imaging.

Electron microscopy (EM)

Fly ovaries were prepared by fixation in 2% glutaraldehyde, post-fixation in 2% osmium tetroxide, dehydration in an ethanol series, then embedding in Epon 812. Ultrathin sections (60 nm) were examined with a JEOL JEM2010 transmission electron microscope operated at an acceleration voltage of 80 kV.

Image analysis of PCD and GSC division

Presence of fluorescent labeling for either a PCD or a GSC division event was visually identified and counted using epifluorescence at 600x magnification using Olympus Fluoview 1000 Confocal microscope.

Later stage PCD was assessed with Hoechst (1 μg/ml) staining indicating chromatin condensation of the DNA. One of the hallmarks of PCD in the germline is nuclear condensation in the nurse cells of pre-vitellogenic egg chambers (40, 41). These condensed nuclei stain brightly and are easily distinguished from normal nuclei (fig. S4). At the PCD checkpoint in the germarium the nuclei are smaller and closer to each other when compared to the nurse cells later in oogenesis. Therefore, rather than assessing chromatin condensation with Hoechst staining, we assessed the level of PCD with the TUNEL assay (terminal deoxyribonucleotidyl transferase dUTP nick end labeling) and the vital dye acridine orange.

Representative images of PCD events and GSC division were acquired using a FV1000 confocal microscope (Olympus). For each condition and experimental run, approximately 10 ovarioles from 10 flies were randomly selected and scored for presence or absence of each label. Each experiment was performed three times except for Hoechst staining (later stage PCD), which was performed four times.

Statistical analysis

Where statistical significance was assessed in this paper, methods based on logistic regression were used for all but two analyses. A regression-based approach is more appropriate here than, for example, simple t-tests, because it allows us to account for

sources of variability in our data that are not directly of interest but nevertheless possibly present. If unaccounted for, these sources of variability can lead to inaccurate assessment of the associations of interest to us, through overly optimistic or pessimistic calculation of p-values. Logistic regression, in turn, is more appropriate here than standard regression because our outcome variables are of a binary nature (e.g., GSCs "divided" or "not") rather than a continuous one. Logistic regression is a statistical method that was originally developed for epidemiological research but is now broadly applied in, for example, ecology (42, 43), medicine (44), and bioinformatics (45), as well as a variety of other fields outside of biology. See (46) for a comprehensive overview of the methodology.

In logistic regression, the odds ratio of an event indicated by the outcome variable (i.e., dependent variable), is modeled as a linear combination of input variables (i.e., independent variables) on a logarithmic scale. The extent to which each independent variable is associated with the dependent variable, controlling for the effects of all other independent variables, is captured in the corresponding regression coefficient. A value of zero for the coefficient corresponds to a lack of association of the independent variable with the dependent variable, controlling for the other independent variables. All statistical p-values reported here under logistic regression models correspond to formal tests of whether or not the coefficient relevant to the hypothesized effect is equal to zero, and were determined using likelihood-based methods and the standard asymptotic chi-square approximation (47). Qualitatively similar results were obtained from a more computationally intensive nonparametric method of p-value calculation, based on exact logistic regression (48). Analyses based on naïve usage of t-tests or ANOVA were run as well, purely for comparison purposes, and yielded, in many cases, even more significant results than those we have quoted for logistic regression. However, we have cited only the logistic regression results throughout the paper, given that these methods are better matched to the binary nature of our outcome variables.

The logistic regressions examining stem cell division and programmed cell death (Fig. 2, fig S3, S4 and table S2-S4) specified a single independent variable representing the effect of interest (e.g., W+ versus W-) and additionally a set of independent variables controlling for possible differences in experimental runs. The logistic regression used in the female HN/LN experiment (Fig. 3 and table S5) specified only a single independent variable (W+ versus W-). However, to be more accurate, this analysis also took into consideration the source of the GSCN and GSC being analyzed. Potential correlations within the same fly were accounted through a standard generalization of logistic regression to correlated observations based on an exchangeable covariance model (49). The first two classes of models were fit using the *glm* function, and the third class, using the *gee* function, in the R software package. The exact logistic regression analyses referred to above were fit using the *elrm* function in R.

Statistical significance for the analysis of *Wolbachia* density in the GSCN and the GSC (fig S6) was assessed using a standard Mann-Whitney U-test. The differences of egg production between infected and non-infected were analyzed using standard *t*-test.

SOM Text

Text S1. Further considerations of systemic and stem cell intrinsic factors on *Wolbachia*-driven alteration of stem cell division

Model 1 proposes a mechanism of *Wolbachia*'s upregulation of stem cell division at the systemic level. A *Wolbachia* infection throughout the body could alter systemic factors known to be relevant for germline stem cell biology. For example, *Drosophila* insulin-like peptides (DILPs) produced in the fly brain are well-described systemic regulators of GSC division (50). *Drosophila melanogaster* mutants in the insulin/IGF signaling pathway display phenotypic differences between infected and non-infected flies (51). *Wolbachia* has been found to infect the brain of *D. melanogaster* adults (52, 53). In *D. mauritiana*, Veneti and collaborators have shown that *Wolbachia* accumulates in the head region during embryonic development (54). In agreement, we found *Wolbachia w*Mau infecting the head of the *D. mauritiana* adult. Infection is distributed throughout the whole brain, including the region described in *D. melanogaster* to contain the median neurosecretory cells (fig. S5), where insulin-like peptides are produced (55). However, it has not been determined if *Wolbachia w*Mau is specifically infecting the neurosecretory cells in *D. mauritiana*. We also found *Wolbachia w*Mau infection in other somatic tissues, including Malpighian tubules, gut, fat bodies and hemolymph.

An additional elaboration of the mechanisms proposed by model 2 could be distinguished between two possibilities: *Wolbachia*'s presence in the niche could directly signal upregulation of GSC division. Alternatively, there is the possibility of *Wolbachia* driving higher GSC division from within the GSC itself. Since stem cell activity is also modulated by stem cell intrinsic factors (56), *Wolbachia* could upregulate GSC division by manipulating these intrinsic factors. To address the latter possibility, we determined if the *Wolbachia* concentration inside the GSCs varied according to the adjacent niche (fig S6). From the density measurements (Sup. Material), as well as clearly illustrated by Figure 3, there are no significant differences in the levels of *Wolbachia* inside the GSC residing next to either type of niches, HN and LN (fig. S6, and compare *Wolbachia* levels at GSCs in Fig. 3A to 3B). Moreover, the fold difference in density between both types of GSCs is only 1.4 (fig. S6).

Although this quantitative image analysis of the levels of *Wolbachia* density in the GSCs shows a slightly higher *Wolbachia* concentration in the GSCs residing at highly infected niches, suggesting that *Wolbachia* in the GSCN could contribute to *Wolbachia* accumulation in the GSCs, this difference was not statistically significant. This greatly contrasts with our analysis measuring the levels of *Wolbachia* in the niches themselves. Detection of *Wolbachia* in niches classified as LN, when compared to niches classified as HN, was reduced by a factor of at least 10 fold, with a highly significant statistical difference (fig. S6, and compare *Wolbachia* levels at GSCNs in Fig. 3A to 3B). These results suggest a mechanism in which *Wolbachia* modulation of GSC activity is performed via the niche, although the participation of stem cell intrinsic factors still remains to be investigated.

Text S2 Evolutionary forces and the cellular mechanisms subverted by *Wolbachia* to enhance their transmission:

Although remarkable, it is not surprising that *Wolbachia* is simultaneously manipulating these two developmental events of oogenesis. Both are key determinants on the rate of egg production, a significant factor for successful *Drosophila* reproduction and *Wolbachia* vertical transmission. During the evolution of this symbiotic interaction, mutations that interfere with any of these two processes are under strong selective pressure.

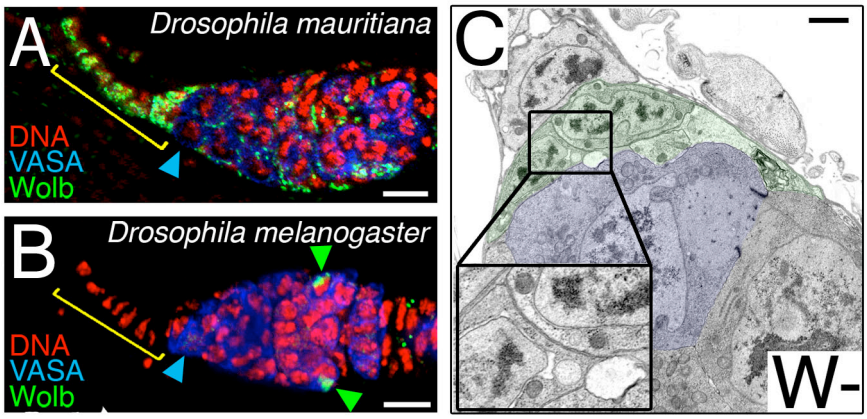

Fig. S1.*Wolbachia* target the GSCN in *D. mauritiana*

(A and B) Diversity of stem cell niche tropism. *Wolbachia* (stained in green) target the GSCN (yellow bracket) in *D. mauritiana* (B) and the SSCN (green arrowheads) in *D. melanogaster* (C). Vasa labels the germline in blue and the blue arrowheads point to the GSCs. Scale bar = 10 µm. (C) Electron micrographs of a germarium from a non-infected *D. mauritiana*. The cap cells are false colored in green and the GSC in blue. The inset in C shows a magnified view of the cytoplasm of the GSCN (cap cells). The cytoplasm of *Wolbachia* infected cap cells (Fig. 1B, main text) looks dramatically different compared to non-infected cap cells (C). Scale bar = 1 µm.

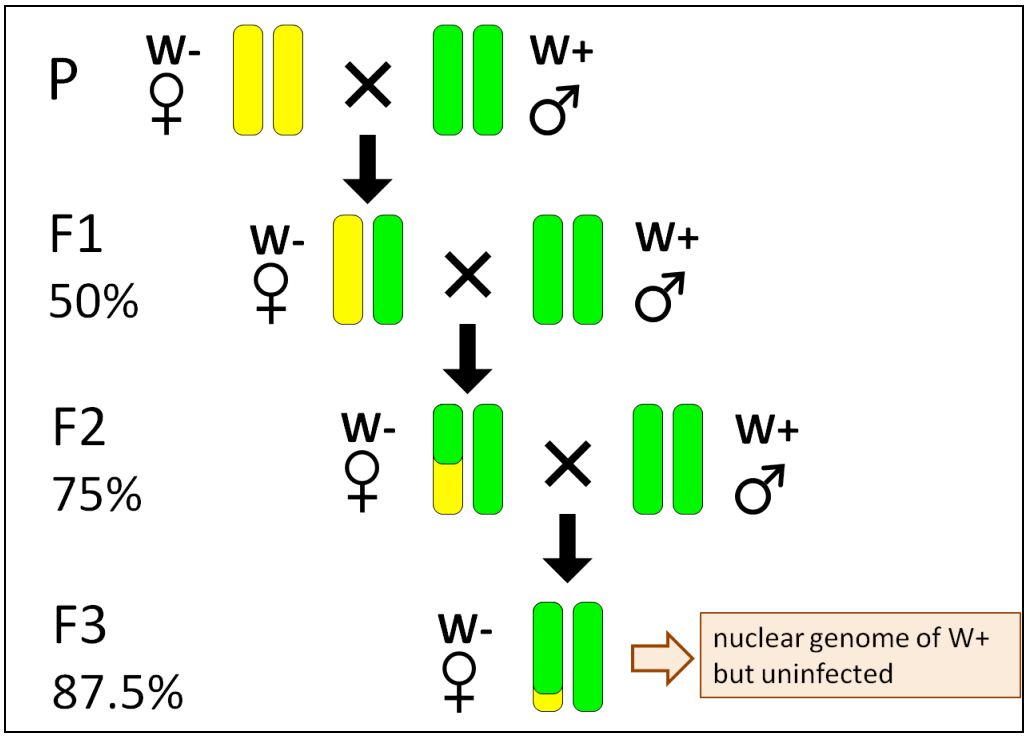

Fig. S2. Backcrosses to infected males homogenize the nuclear genome but leave the *Wolbachia* infection status unchanged.

The backcrossing scheme is illustrated in the above figure by visualizing fly chromosomes as differentially colored rods. Antibiotic curing creates a selective pressure that can have phenotypic consequences (34). Potentially, the treatment could cause differences in stem cell division and programmed cell death (PCD). To counteract potential selection during antibiotic treatment of infected flies, crosses to infected males from the original stock were performed. Uninfected female flies (W-) obtained from the antibiotic treatment were crossed to infected male flies. This cross was repeated for two more generations so that the final nuclear genetic background of the uninfected (W-) and infected stock (W+) was about 90% similar. Since *Wolbachia* is only maternally transmitted, the progeny from every cross is not infected and the infection status of the males is irrelevant in this regard.

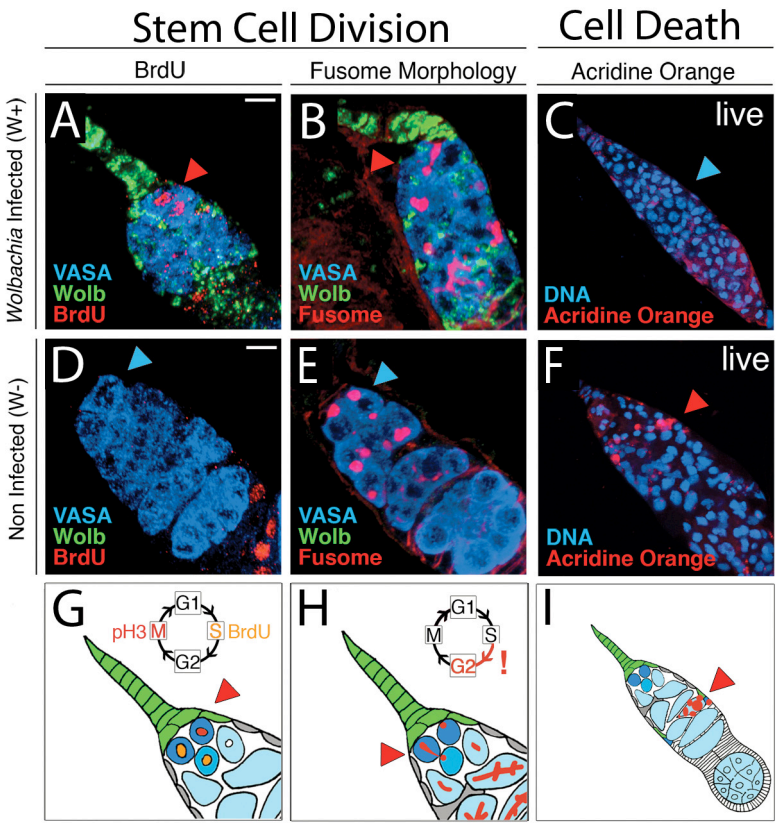

Fig. S3. *Wolbachia* infection increases GSC mitotic activity and suppresses PCD.
Confocal images of *D. mauritiana* germaria infected [W+, (**A-C**)] and non-infected [W-, (**D-F**)]. Representative images for the respective infection status are shown. The presence (red arrow head) or absence (blue arrowhead) of the event being measured is indicated (A-F). Germline stem cell division (A-B; D-E) and cell death (C-F) were analyzed using markers indicated on the top of the figure and in the legend of each panel. Scale bar = 10µm. (**G**, **H** and **I**) Indicate the cell cycle specificity or morphological features for all the markers utilized in this study, including Fig. 3 (see text for details).

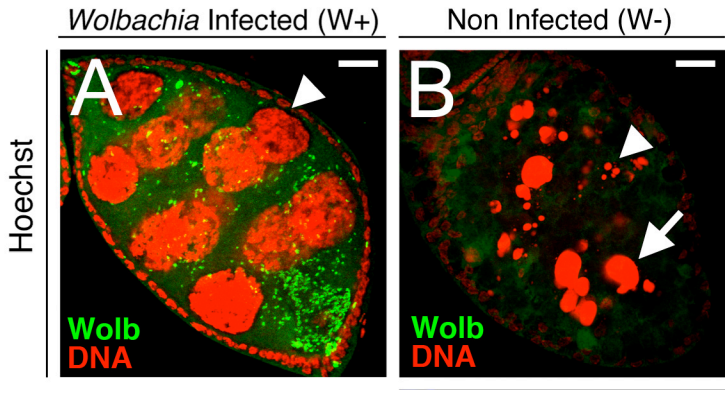

Fig. S4. *Wolbachia* have no effect on programmed cell death (PCD) in previtellogenic egg chambers.

Cell death was assessed by DNA morphology indicated by the nuclear dye Hoechst (red). (A) Healthy egg chamber, indicated by evenly dispersed chromatin in the nurse cells (arrowhead), from a *Wolbachia* infected *D. mauritiana* fly. (B) Highly condensed (arrow) and fragmented DNA (arrowhead) is a sign of a dying egg chamber from a non-infected *D. mauritiana* fly. No significant differences in cell death were detected between infected and non-infected egg chambers in previtellogenic stages (see table S3). Scale bar = 20µm

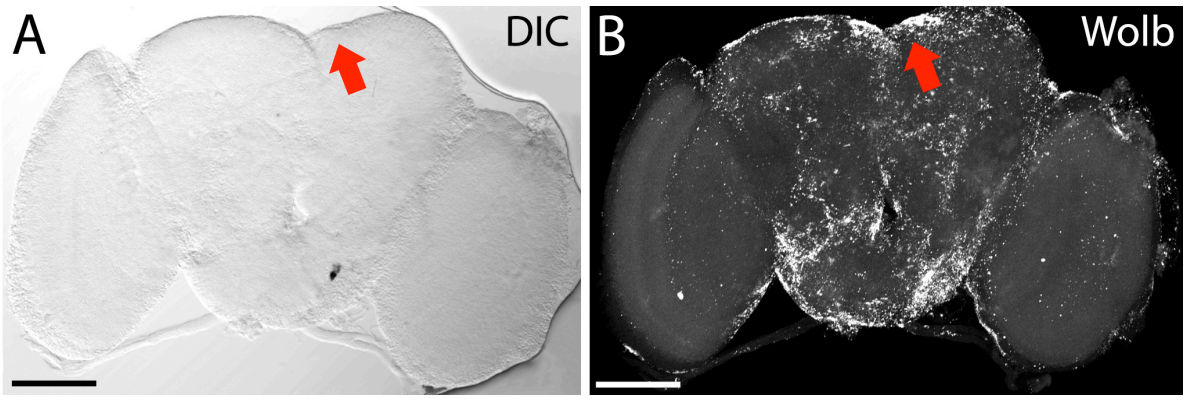

Fig. S5. *Wolbachia* potentially localize to the cells in the brain that secrete insulin-like peptides.

Representative image of *Wolbachia* localization in the brain of *Drosophila mauritiana*. Figure (A) shows a differential interference contrast image (DIC) of the immunofluorescence image in (B). Red arrow points to the region containing the median neurosecretory cells (mNSC), which are thought to secrete insulin-like peptides (57). Maximum projection of a whole brain (B) shows that *Wolbachia* is distributed throughout the whole brain but there is consistent accumulation in the general area containing the mNSCs (n= 10 brains). Scale bar = 100μm.

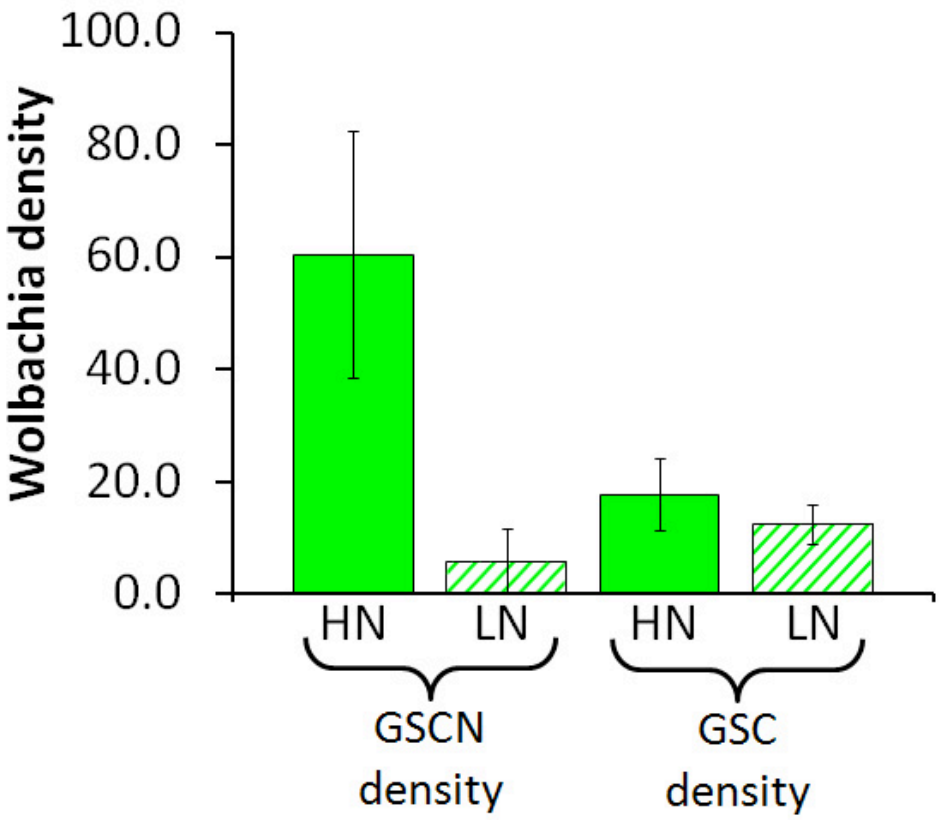

Fig. S6. The difference in *Wolbachia* density in GSCNs is substantially larger than in GSCs and these niches can be classified as highly infected (HN) and infected with low levels (LN).

Germline stem cell niches of *Wolbachia* infected flies were classified either as HN (for GSCNs with high *Wolbachia* infection) or LN (GSCNs with low *Wolbachia* infection). Tridimensional confocal image analysis was performed to quantify *Wolbachia* density in HN and LN GSCNs and adjacent GSCs. All images were obtained using identical acquisition parameters. *Wolbachia* density is shown in arbitrary values. The intensity of the *Wolbachia* signal from each pixel was summed and divided by the total number of pixels within the area delimitated by the GSCN and GSC. The *Wolbachia* signal in niches classified as LN was reduced by a factor of at least 10 when compared to niches classified as HN, the error bars correspond to standard deviation. The difference between *Wolbachia* density of HN and LN GSCNs is statistically significant ($P = 1.6 \times 10^{-7}$, Mann–Whitney U test, N=23) whereas the difference of *Wolbachia* density in GSCs abutting HN and LN niches is not ($P = 0.077$, Mann–Whitney U test, N=23).

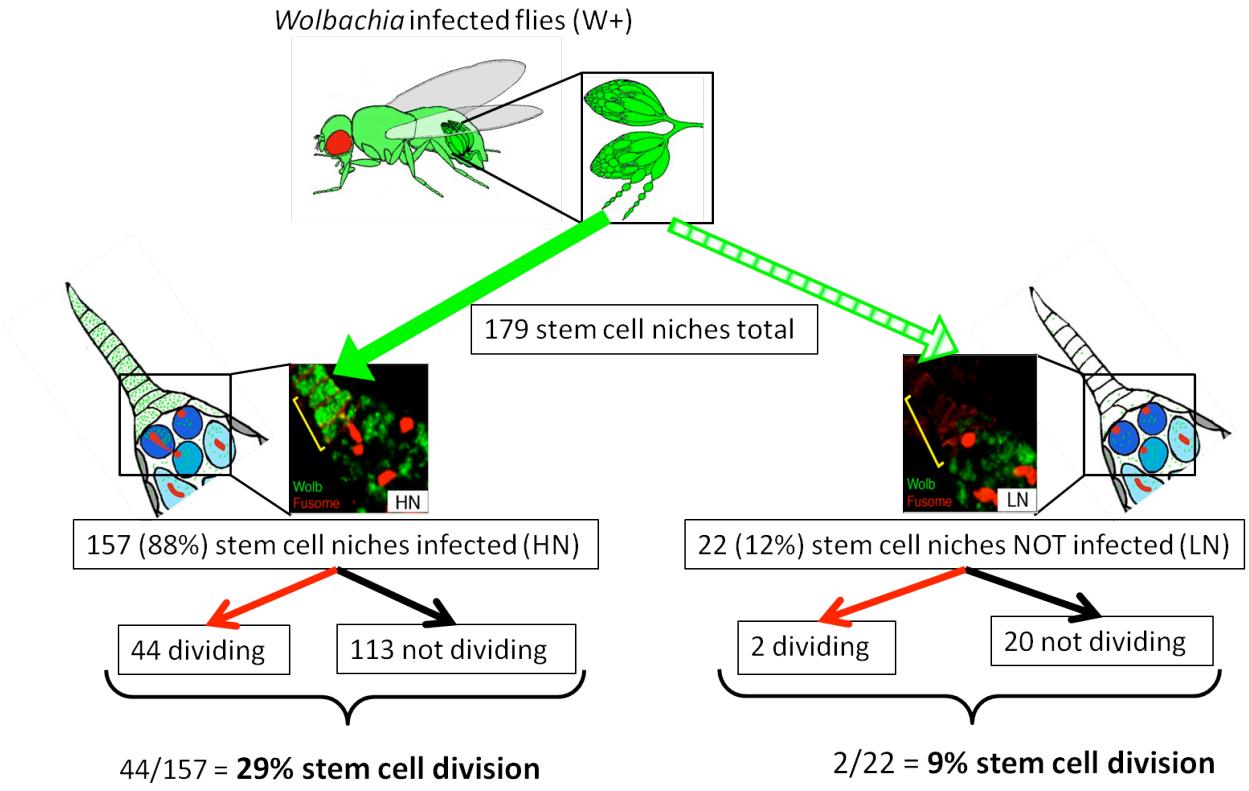

Fig. S7. Occasional non-*Wolbachia* infected niches (LN) allow the assessment of niche dependent stem cell division within the same fly *in vivo*.

Schematic of the experiments shown in Figure 3. The numbers shown here correspond to experiment one. Ovaries from *Wolbachia* infected *Drosophila mauritiana* (W+) were dissected, and after staining for cell division markers (BrdU or Fusome), germaria were divided into two groups according to the levels of infection in the germline stem cell niche (HN and LN, see fig. S6). The frequency of stem cell division was then assessed for each group separately (Results Fig. 3). In this experiment, 29% of the stem cells neighboring niches with high levels of *Wolbachia* were undergoing germline stem cell division. This contrasts with only 9% of stem cell division in the niches that were not infected with *Wolbachia* (LN).

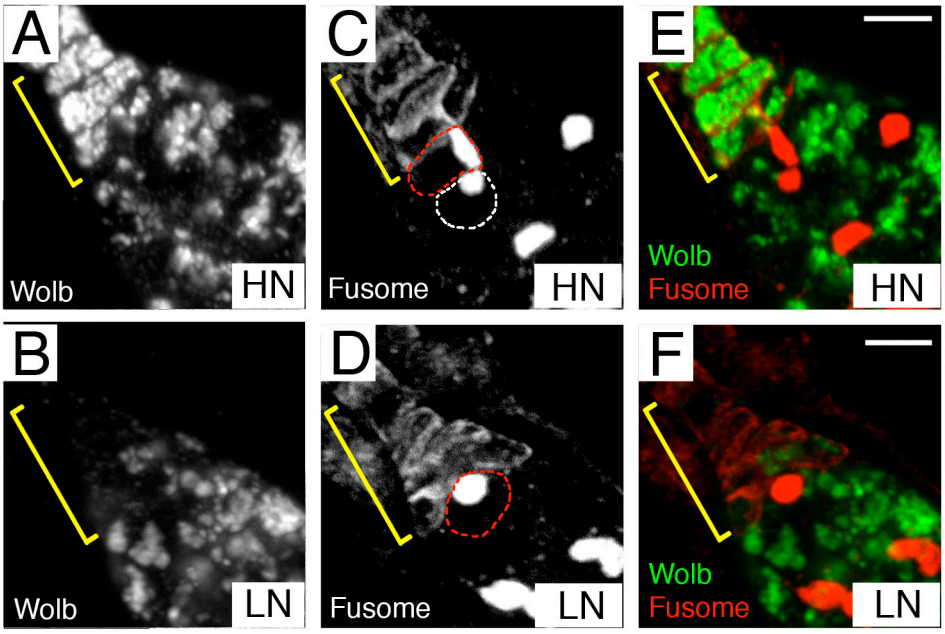

Fig. S8. Different levels of GSCN infection in the same infected fly.

Germaria from infected *D. mauritiana*. Red dotted circles indicate the GSCs (C and D). Cap cells and the base of the terminal filament indicated by yellow brackets (A-F). In infected flies, most of the GSCN show high levels of *Wolbachia* (HN, see A and E). Occasionally in infected flies, a few germaria have niches harboring low levels of *Wolbachia* (LN, see B and F). (A, C and E) The *Wolbachia*-rich niche (HN) is adjacent to a dividing GSC and its respective daughter cell (white dotted circle in C). (B, D and F) The *Wolbachia*-poor niche (LN) harbors a non-dividing GSC. Scale bar = 5μm.

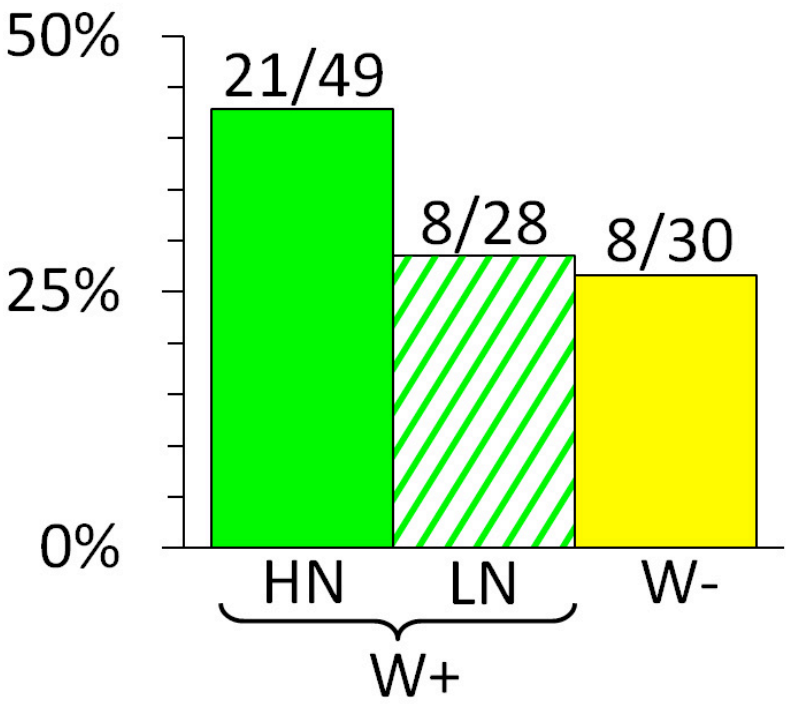

Fig. S9. GSC division in *Drosophila mauritiana* testes.

Typically, in infected *Drosophila mauritiana* males, 64% of the hubs were highly infected with *Wolbachia* (Fig 3F, N=77, table S6). In these niches (HN), the ratio of dividing stem cells per testis was 43 % (assessed by pH3) higher than in niches with low *Wolbachia* levels (LN), but not statistically significant ($P = 0.34$, logistic regression). The proportion of dividing GSCs per testis in LN niches was with 29% (Fig. 3G) more similar to non-infected (W-) testes (27%, table S6, $P = 0.89$, logistic regression).

Table S1. Fecundity of *Wolbachia* infected flies (W+) and non-infected flies (W-)

Average (mean) egg production per female ± standard deviation in *Wolbachia* infected flies (W+) and non-infected flies (W-). Experiment A was carried out at room temperature (RT) over a total period of 46 days and experiment B was carried out at 25°C with controlled humidity (60%) over 20 days. For comparison the average fecundity of experiment A over 20 days is also indicated. Averages and standard deviation were obtained from 3 independent samples, each containing 5 females for both W+ and W-. Fold difference is the ratio of the average egg production in W+ females to that in W- females. The *P*-values were calculated using an unpaired, one-tailed Student's *t*-test.

	W+	W-	Fold difference (W+/W-)	*P* - value
Experiment A – RT (20 days)	216 ± 29	62 ± 16	3.5	6.5×10^{-4}
Experiment A - RT (46 days)	305 ± 42	74 ± 11	4.1	3.9×10^{-4}
Experiment B - 25°C (20 days)	61 ± 24	15 ± 8	4.1	1.7×10^{-2}

Table S2. Germline stem cell (GSC) division

Wolbachia infection consistently and significantly increases the mitotic activity of GSCs. The frequency of dividing GSCs of *Wolbachia* infected (W+) and non-infected (W-) is shown for each experiment as a percent of percent of germaria with a dividing GSC. Quantification of GSC division was measured in 9 independent experiments (three each using BrdU, Fusome and Phospho-histone H3). The number of germaria analyzed (n) is given in parentheses. Fold difference is the percent of germaria with a dividing GSC in W+ females divided by the percent germaria with a dividing GSC in W- females. The overall average of stem cell division in W+ is 2.1 (± 0.65) fold of W-, n = 2377, logistic regression: $P_{pH3} = 5.4 \times 10^{-3}$, $N = 621$; $P_{BrdU} = 2.0 \times 10^{-2}$, $N = 1061$; $P_{Fusome} = 4.3 \times 10^{-3}$.

	Percentage of GSC division in W+ (n)	Percentage of GSC division in W- (n)	Fold difference (W+/W-)
Phospho-histone H3 #1	8.8% (80)	3.5% (85)	2.5
Phospho-histone H3 #2	10% (101)	4.0% (101)	2.8
Phospho-histone H3 #3	6.6% (121)	2.3% (133)	2.9
Average pH3 (± stdv)	**8.8% (± 2.1%)**	**3.3% (± 0.88%)**	**2.7 (± 0.22)**
BrdU #1	9.2% (98)	6.3% (126)	1.5
BrdU #2	4.4% (180)	1.6% (190)	2.8
BrdU #3	7.1% (240)	3.5% (227)	2.0
Average BrdU(± stdv)	**6.9% (± 2.4%)**	**3.8% (± 2.4%)**	**2.1 (± 0.68)**
Fusome #1	28% (100)	19% (100)	1.3
Fusome #2	22% (111)	16% (189)	1.3
Fusome #3	35% (100)	17% (95)	2.0
Average Fusome (± stdv)	**28% (± 6.5%)**	**17% (± 1.4%)**	**1.6 (± 0.41)**

Note: To evaluate if differences in the frequency of stem cell division are not due to different amounts of GSCs in W+ and W- ovaries, we assessed the average number of GSCs per germarium for both conditions using Fusome and Vasa staining: the average number of GSCs per germarium is comparable between W+ (2.73 ± 0.78, $N = 100$ germaria) and W- (2.41 ± 0.66, $N = 97$ germaria).

Table S3. Programmed Cell Death (PCD) in previtellogenic egg chambers

Wolbachia's presence has no significant effect on programmed cell death (PCD) in previtellogenic egg chambers in *Drosophila mauritiana*. Each row of table 3 (Hoechst #1 - #4) refers to one independent experiment. Percent of egg chambers undergoing programmed cell death (PCD) of *Wolbachia* infected flies (W+) and non-infected flies (W-) for each experiment. Fold difference is the percent egg chambers undergoing PCD in W+ females divided by the percent egg chambers undergoing PCD in W- females. The number (n) of egg chambers analyzed is given in parentheses. The total n = 1175, logistic regression: $P = 4.3 \times 10^{-1}$

	Percentage of PCD in W+ (n)	Percentage of PCD in W- (n)	Fold difference (W+/W-)
Hoechst #1	3.9% (51)	7.6% (53)	0.52
Hoechst #2	8.7% (103)	6.7% (104)	1.3
Hoechst #3	16% (222)	20% (211)	0.77
Hoechst #4	12% (225)	12% (206)	1.0
Average (± stdv)	**10% (± 5.1%)**	**12% (± 5.3%)**	**0.90 (± 0.33)**

Table S4. Programmed Cell Death (PCD) in the germarium.

Wolbachia infection reduces the frequency of PCD in the germarium by approximately half. Each row of table 4 (TUNEL/Apotag #1 - #3 and Acridine Orange #1 - #3) refers to one independent experiment. Percent of germaria undergoing programmed cell death (PCD) of *Wolbachia* infected flies (W+) and non-infected flies (W-) for each experiment. The number (n) of germaria analyzed for TUNEL and Acridine Orange are given in parentheses. Fold difference is the percent germaria undergoing PCD in W+ females divided by the percent germaria undergoing PCD in W- females. The overall average of PCD in the W+ germarium is 0.56 (± 0.20) fold of W-, n = 1556 germaria, logistic regression: $P_{\text{TUNEL}} = 8.0 \times 10^{-3}$, $N = 802$; $P_{\text{Acridine Orange}} = 1.2 \times 10^{-7}$.

	Percentage of PCD in W+ (n)	Percentage of PCD in W- (n)	Fold difference (W+/W-)
TUNEL/Apotag #1	3.7% (109)	5.0% (121)	0.74
TUNEL/Apotag #2	8.9% (101)	26% (102)	0.34
TUNEL/Apotag #3	17% (170)	20% (199)	0.82
Average TUNEL (± stdv)	**9.7% (± 6.5%)**	**17% (± 11%)**	**0.63 (± 0.26)**
Acridine Orange (AO) #1	11% (100)	18% (80)	0.63
Acridine Orange (AO) #2	9.4% (127)	37% (108)	0.26
Acridine Orange (AO) #3	20% (164)	33% (175)	0.59
Average AO (± stdv)	**13% (± 5.4%)**	**29% (± 10%)**	**0.49 (± 0.20)**

Table S5. Germline stem cell (GSC) division in highly infected GSCN (HN) and GSCN with low infection (LN).

Total number (percentages in parentheses) of germaria with niches having high *Wolbachia* infection (HN) and niches having low *Wolbachia* infection (LN**).** Absolute number and percentage of germaria with high infection of the GSCN (HN). The GSCN was identified by morphology, cortical spectrin staining and absence of the germline marker VASA at the anterior most tip of the germarium. GSC division in Exp 1 and Exp 2 was assessed using Fusome staining, in Exp 3 and Exp 4 using BrdU. Presence of dividing GSCs was assessed in HN and LN germaria. Logistic regression: $P = 2.4 \times 10^{-2}$ (HN vs LN)

Experiment	Total germaria	HN (%)	HN with dividing GSC (%)	LN (%)	LN with dividing GSC (%)
Exp 1	179	157 (88%)	46 (29%)	22 (12%)	2 (9.1%)
Exp 2	189	187 (99%)	40 (21%)	2 (1.1%)	0
Exp 3	240	203 (85%)	16 (7.9%)	37 (15%)	1 (2.7%)
Exp 4	180	170 (94%)	8 (4.7%)	10 (5.6%)	0

Table S6. Germline stem cell (GSC) division in the testes.

Total number of testes with germline stem cell niches (GSCNs) having high *Wolbachia* infection (HN) and niches having low *Wolbachia* infection (LN) with corresponding number and percentage (in parenthesis) of dividing germline stem cells (GSCs). Percentage of GSCs dividing represents the ratio of dividing GSCs to the number of GSCNs analyzed. Logistic regression: $P = 0.34$ (HN vs LN), $P = 0.26$ (HN vs W-), $P = 0.89$ (W- vs LN)

	Number of Testes	Number of dividing GSC (%)
W+ (HN)	49	21 (43%)
W+ (LN)	28	8 (29%)
W-	30	8 (27%)

Movie S1

Rotation of 3D reconstruction of infected *D. mauritiana* germarium confocal sectioning. DNA is in blue and *Wolbachia* is in green. The nuclear envelope of the GSCN and stalk cells is labeled in red. DNA staining is shown only on the first frame to facilitate *Wolbachia* visualization. Note that the terminal filament and the cap cells (GSCN, leftmost structure) harbor the highest concentration of *Wolbachia*.

Supporting references

32. Paraskevopoulos, C., et al., *Toward a Wolbachia multilocus sequence typing system: discrimination of Wolbachia strains present in Drosophila species.* Curr Microbiol, 2006. **53**(5): p. 388-95.
33. Giordano, R., S.L. O'Neill, and H.M. Robertson, *Wolbachia infections and the expression of cytoplasmic incompatibility in Drosophila sechellia and D. mauritiana.* Genetics, 1995. **140**(4): p. 1307-17.
34. Bordenstein, S.R. and J.H. Werren, *Do Wolbachia influence fecundity in Nasonia vitripennis?* Heredity, 2000. **84**(Pt 1): p. 54-62.
35. Drummond-Barbosa, D. and A.C. Spradling, *Stem cells and their progeny respond to nutritional changes during Drosophila oogenesis.* Dev Biol, 2001. **231**(1): p. 265-78.
36. Frydman, H.M. and A.C. Spradling, *The receptor-like tyrosine phosphatase lar is required for epithelial planar polarity and for axis determination within drosophila ovarian follicles.* Development, 2001. **128**(16): p. 3209-20.
37. Frydman, H.M., et al., *Somatic stem cell niche tropism in Wolbachia.* Nature, 2006. **441**(7092): p. 509-12.
38. Dubreuil, R., et al., *Drosophilia spectrin. I. Characterization of the purified protein.* J Cell Biol, 1987. **105**(5): p. 2095-102.
39. Lilly, M.A. and A.C. Spradling, *The Drosophila endocycle is controlled by Cyclin E and lacks a checkpoint ensuring S-phase completion.* Genes Dev, 1996. **10**(19): p. 2514-26.
40. McCall, K., *Eggs over easy: cell death in the Drosophila ovary.* Dev Biol, 2004. **274**(1): p. 3-14.
41. Buszczak, M. and L. Cooley, *Eggs to die for: cell death during Drosophila oogenesis.* Cell Death Differ, 2000. **7**(11): p. 1071-4.
42. Trexler, J.C. and J. Travis, *Nontraditional Regression-Analyses.* Ecology, 1993. **74**(6): p. 1629-1637.
43. Peeters, E.T.H.M. and J.J.P. Gardeniers, *Logistic regression as a tool for defining habitat requirements of two common gammarids.* Freshwater Biology, 1998. **39**(4): p. 605-615.
44. Bagley, S.C., H. White, and B.A. Golomb, *Logistic regression in the medical literature: Standards for use and reporting, with particular attention to one medical domain.* Journal of Clinical Epidemiology, 2001. **54**(10): p. 979-985.

45. Liao, J.G. and K.V. Chin, *Logistic regression for disease classification using microarray data: model selection in a large p and small n case.* Bioinformatics, 2007. **23**(15): p. 1945-1951.
46. Hosmer, D.W. and S. Lemshow, *Applied Logistic Regression*. 2nd ed. 2000, New York: John Wiley & Sons.
47. Hosmer, D.W. and S. Lemshow, *"Testing for the significance of the model" in Applied Logistic Regression*. 2nd ed. 2000, New York: John Wiley & Sons. Ch 2.4, p. 36-39.
48. Hosmer, D.W. and S. Lemshow, *"Exact Methods for Logistic Regression Models" in Applied Logistic Regression*. 2nd ed. 2000, New York: John Wiley & Sons. Ch 8.4, p. 330-338.
49. Hosmer, D.W. and S. Lemshow, *"Logistic Regression Models for the Analysis of Correlated Data" in Applied Logistic Regression*. 2nd Edition ed. 2000, New York: John Wiley & Sons. Ch 8.3, p. 308-329.
50. LaFever, L. and D. Drummond-Barbosa, *Direct control of germline stem cell division and cyst growth by neural insulin in Drosophila.* Science, 2005. **309**(5737): p. 1071-3.
51. Gronke, S., et al., *Molecular evolution and functional characterization of Drosophila insulin-like peptides.* PLoS Genet, 2010. **6**(2): p. e1000857.
52. Min, K.T. and S. Benzer, *Wolbachia, normally a symbiont of Drosophila, can be virulent, causing degeneration and early death.* Proc Natl Acad Sci U S A, 1997. **94**(20): p. 10792-6.
53. Albertson, R., et al., *Symmetric and asymmetric mitotic segregation patterns influence Wolbachia distribution in host somatic tissue.* J Cell Sci, 2009. **122**(Pt 24): p. 4570-83.
54. Veneti, Z., et al., *Heads or tails: host-parasite interactions in the Drosophila-Wolbachia system.* Appl Environ Microbiol, 2004. **70**(9): p. 5366-72.
55. Ikeya, T., et al., *Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in Drosophila.* Curr Biol, 2002. **12**(15): p. 1293-300.
56. Hatfield, S.D., et al., *Stem cell division is regulated by the microRNA pathway.* Nature, 2005. **435**(7044): p. 974-8.
57. Broughton, S.J., et al., *Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands.* Proc Natl Acad Sci U S A, 2005. **102**(8): p. 3105-10.